

Appl. No. : 09/331,631
Filed : June 21, 1999

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

Claim 1 (previously amended): An isolated or purified protein fragment having antimicrobial activity, wherein said protein fragment is a polypeptide comprising a cysteine spacing of C-3X-C-nX-C-3X-C (SEQ ID NOS: 38 and 39) wherein n is 11 or 12, X is any amino acid residue other than cysteine, and C is cysteine.

Claim 2 (previously amended): An isolated or purified protein comprising at least one polypeptide fragment according to claim 1, wherein said polypeptide fragment has a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 5.

Claim 3 (previously amended): An isolated or purified protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5.

Claims 4-10 (cancelled)

Claim 11 (previously amended): A composition comprising the protein fragment of claim 1 together with an agriculturally-acceptable carrier diluent or excipient.

Claim 12 (withdrawn): A composition comprising an antimicrobial protein according to claim 1 together with a pharmaceutically-acceptable carrier diluent or excipient.

Claim 13 (currently amended): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the protein fragment of claim 1 for a period sufficient to reduce the number of said microbes, wherein said protein fragment is a polypeptide comprising a cysteine spacing of C-3X-C-nX-C-3X-C (SEQ ID NOS: 38 and 39) wherein n is 11 or 12, X is any amino acid residue other than cysteine, and C is cysteine.

Claim 14 (withdrawn): A method of controlling microbial infestation of a mammal, said method comprising treating the mammal with an antimicrobial protein according to claim 1.

Claim 15 (withdrawn): The method of claim 14, wherein said mammal is a human.

Claim 16 (currently amended): A method of preparing an antimicrobial protein, said method comprising:

a) searching a sequence database using a suitable algorithm to identify an amino acid sequence which forms a helix-turn-helix structure or designing an amino acid sequence which forms a helix-turn-helix structure;

Appl. No. : 09/331,631
Filed : June 21, 1999

b) substituting individual residues in said amino acid sequence to achieve a sequence having the same distribution of positively charged residues and cysteine residues as the distribution found in a protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5;

c) synthesizing chemically or expressing by recombinant DNA techniques in liquid culture ~~an antimicrobial~~ a protein comprising said substituted amino acid sequence; and

d) isolating and testing said antimicrobial protein to confirm that said protein has antimicrobial activity.

Claim 17 (previously amended): The protein fragment of claim 1, wherein said protein fragment is a polypeptide comprising a cysteine and tyrosine or phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z (SEQ ID NOS: 34-36) wherein X and C are as defined in claim 1, and Z is tyrosine or phenylalanine.

Claim 18 (previously amended): The protein fragment of claim 1, wherein said cysteine spacing comprises C-2X-C-3X-C-(10-12)X-C-3X-C-3X-C (SEQ ID NOS: 31-33) wherein X and C are as defined in claim 1.

Claim 19 (previously amended): An isolated or purified protein fragment having antimicrobial activity, wherein said protein fragment is selected from the group consisting of:

- residues 29 to 73 of SEQ ID NO: 1
- residues 74 to 116 of SEQ ID NO: 1
- residues 117 to 185 of SEQ ID NO: 1
- residues 186 to 248 of SEQ ID NO: 1
- residues 29 to 73 of SEQ ID NO: 3
- residues 74 to 116 of SEQ ID NO: 3
- residues 117 to 185 of SEQ ID NO: 3
- residues 186 to 248 of SEQ ID NO: 3
- residues 33 to 75 of SEQ ID NO: 5
- residues 76 to 144 of SEQ ID NO: 5
- residues 145 to 210 of SEQ ID NO: 5
- residues 34 to 80 of SEQ ID NO: 7
- residues 81 to 140 of SEQ ID NO: 7
- residues 33 to 79 of SEQ ID NO: 8

Appl. No. : 09/331,631
Filed : June 21, 1999

F1
Contd.

residues 80 to 119 of SEQ ID NO: 8
residues 120 to 161 of SEQ ID NO: 8
residues 32 to 91 of SEQ ID NO: 21
residues 25 to 84 of SEQ ID NO: 22
residues 29 to 94 of SEQ ID NO: 24; and
residues 31 to 85 of SEQ ID NO: 25.

Claim 20 (previously amended): The protein fragment of claim 1 which is truncated, wherein said truncated protein fragment retains the antimicrobial activity of the nontruncated protein fragment.

Claim 21 (previously amended): A homologue of any of the protein fragments of claim 1.

Claims 22-29 (cancelled)

Claim 30 (previously amended): A composition comprising the protein fragment of claim 19 together with an agriculturally-acceptable carrier diluent or excipient.

Claim 31 (previously amended): A composition comprising the protein fragment of claim 19 together with a pharmaceutically-acceptable carrier diluent or excipient.

Claims 32 and 33 (cancelled)

Claim 34 (withdrawn): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the composition of claim 11 for a period sufficient to reduce the number of said microbes.

Claim 35 (cancelled)

Claim 36 (withdrawn): A method of controlling microbial infestation of a mammal, the method comprising treating the mammal with a composition according to claim 12.

Claim 37 (withdrawn): The method of claim 19, wherein said mammal is a human.

Claim 38 (cancelled)

Claim 39 (withdrawn): A method of controlling microbial infestation of a mammal, the method comprising treating the mammal with a composition according to claim 30.

Claim 40 (withdrawn): The method of claim 39, wherein said mammal is a human.

Claim 41 (previously added): The method of claim 16, further comprising forming disulphide linkages between said cysteine residues.

Claim 42 (cancelled)

Appl. No. : 09/331,631
Filed : June 21, 1999

Claim 43 (previously amended): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the composition of claim 30 for a period sufficient to reduce the number of said microbes.

Claim 44 (currently amended): A method of reducing the number of fungi infesting a plant, the method comprising administering to said plant an effective amount of an antimicrobial protein for a period sufficient to reduce the number of said fungi, wherein:

said antimicrobial protein comprises a polypeptide comprising a cysteine spacing of C-3X-C-(10-12)X-C-3X-C (SEQ ID NOS: 37-39) wherein X is any amino acid residue other than cysteine, and C is cysteine.

Claim 45 (currently amended): A method of reducing the number of fungi infesting a plant, the method comprising administering to said plant an effective amount of a composition comprising an antimicrobial protein together with an agriculturally-acceptable carrier diluent or excipient for a period sufficient to reduce the number of said fungi, wherein:

said antimicrobial protein comprises a polypeptide comprising a cysteine spacing of C-3X-C-(10-12)X-C-3X-C (SEQ ID NOS: 37-39) wherein X is any amino acid residue other than cysteine, and C is cysteine.

Claim 46 (currently amended): A composition comprising the isolated or purified protein of claim 2 together with an agriculturally-acceptable carrier diluent or excipient.

Claim 47 (previously added): A composition comprising the isolated or purified protein of claim 3 together with an agriculturally-acceptable carrier diluent or excipient.

Claim 48 (previously added): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the isolated or purified protein of claim 2 for a period sufficient to reduce the number of said microbes.

Claim 49 (previously added): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the isolated or purified protein of claim 3 for a period sufficient to reduce the number of said microbes.

Claim 50 (previously added): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the protein fragment of claim 17 for a period sufficient to reduce the number of said microbes.

Appl. No. : 09/331,631
Filed : June 21, 1999

Claim 51 (previously added): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the protein fragment of claim 18 for a period sufficient to reduce the number of said microbes.

F-1
C. Conclude
Claim 52 (previously added): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the protein fragment of claim 19 for a period sufficient to reduce the number of said microbes.

Claim 53 (previously added): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the composition of claim 46 for a period sufficient to reduce the number of said microbes.

Claim 54 (previously added): A method of reducing the number of microbes infesting a plant, the method comprising administering to said plant an effective amount of the composition of claim 47 for a period sufficient to reduce the number of said microbes.